Excerpt From:
Diversity of Marine and Freshwater Algal Toxins

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Ciguatera Toxins

One of the seafood intoxications caused by ladder-like polyether toxins is ciguatera fish poisoning. Ciguatera occurs circumglobally in tropical coral reef regions (Figure 5), and results from the consumption of fish which have accumulated toxins through the food web. It is estimated to affect over 50,000 people annually, and is no longer a disease limited to the tropics, due both to travel to the tropics and to shipping of tropical fish species to markets elsewhere in the world (34). Large carnivorous fishes associated with coral reefs are the most frequent source of ciguatera. Baracuda, snapper, grouper, jacks, and moray eel are particularly notorious for their potential to carry high toxin loads. However, smaller herbivorous fishes may also be ciguatoxic, particularly when viscera are consumed. The symptoms of ciguatera vary somewhat geographically, as well as between individuals and incidents, and may also vary temporally within an area, but generally include early onset (2-6 hour) gastrointestinal disturbance, including nausea, vomitting, and diarrhea, and may be followed by a variety of later onset (18 hour) neurological sequelae, including numbness of the perioral area and extremities, reversal of temperature sensation, muscle and joint aches, headache, itching, tachycardia, hypertension, blurred vision, and paralysis. Ciguatera on rare occasions can be fatal. Ciguatera symptoms in the Caribbean differ somewhat from those in the Pacific in that gastrointestinal symptoms dominate, whereas in the Pacific neurological symptoms tend to dominate. This may reflect geographic differences in the toxins involved (35).

The origin of ciguatera toxins has been identified as the benthic coral reef associated dinoflagellate, Gambierdiscus toxicus (36), which grows as an epiphyte on filamentous macroalgae associated with coral reefs and reef lagoons. Its toxins enter the food web when these algae are grazed upon by herbivorous fishes and probably also invertebrates. G. toxicus produces two classes of polyether toxins, the ciguatoxins (CTX) and maitotoxins (MTX). The CTX are lipophilic and are accumulated in fish through food web transfer. More than 20 CTX congeners have been isolated (37); however, only a few have been fully characterized structurally. Three classes of CTX are currently recognized, based on polyether backbone structure (Figure 6). The first CTX to be purified (38) and structrually elucidated (39) is now known as CTX-1, CTX1B, or P-CTX-1 (for Pacific CTX-1). CTX-1 is the parent compound for the type 1 CTX group, which possess 60 carbons, in 13 fused ether rings. CTX-1 is believed to be responsble for 90% of the toxicity associated most of the Pacific intoxications. Pacific CTX-2 and CTX-3 are also type 1 toxins found in fish flesh (40). The toxins found in fish flesh are more highly oxygenated than the congeners isolated from G. toxicus, suggesting that they are metabolites of the dinoflagellate toxins of the same backbone structure. CTX-1, CTX-2 and CTX-3 may all be derived from dinoflagllate precursors, CTX-4A and CTX4B (41). Type 2 CTX congeners possess 57 carbons in 13 fused ether rings. Type 2 ciguatoxins lack the C1-C4 side chain present in type 1 and have an 8, rather than 7 membered ring E. Like the type 1 CTX toxins, there is evidence that CTX2A1, a congener present in fish, represents an oxygenated metabolite of a dinoflagellate precursor, CTX3C (42). Several Caribbean CTX (C-CTX) congeners have been isolated chromatographically, of which the most abundant, C-CTX-1, is the first to be fully

structurally characterized (43). C-CTX-1 represents a third class of ciguatoxin that lacks the spiroketal at C52, which is replaced by an additional fused 6-membered cyclic ether.

The CTX are structurally related to the brevetoxins and compete with brevetoxin for binding bind to site 5 on the voltage dependent sodium channel with a high affinity (Kd ~ 0.04 - 4 nM) (44) . The LD50 (i.p.) in mice for CTX-1 is 0.25 mg/kg (37), whereas the potency of the caribbean toxin C-CTX-1 is ten fold lower (LD50 3.6 mg/g) (35). Various estimates of human toxic potency have been made. A concentration of >0.1 ppb of P-CTX-1 is estimated to be toxic to humans, compared to >1.0 ppb C-CTX-1. In a study of fish implicated in ciguatera cases in French Polynesia, a minimum toxicity level to humans was estimated at 0.5 ng/g (37). Among the CTX congeners, binding affinity correlates well with toxic potency (i.p.) in mice. However, the toxic potency of CTX in mice is several orders of magnitude greater than that of the brevetoxins, relative to their binding affinities at the sodium channel (e.g., for CTX1 and PbTx3, LD50 = 0.25 mg/kg vs >200 mg/kg , whereas KD = 0.04 nM vs 2 nM, respectively). This may be related to differences in the bioavailability of the toxins or to undefined toxic effects of ciguatoxin.

The maitotoxins (Figure 7), like CTX or PbTx, are transfused ladder-like polyether toxins, but are somewhat more polar, due to the presence of multiple sulfate groups. MTX was originally identified as a water soluble toxin in the viscera of surgeonfishes (45), and later found to be the principal toxin produced by Gambierdiscus toxicus. The structure of MTX was first elucidated by Murata et al.(46), with complete stereochemistry resolved by Zheng et al.(47). Three MTX congeners have been identified in Pacific isolates of G. toxicus, MTX-1 and MTX-2 (3422 and 3298 daltons, respectively) and a smaller compound, MTX-3 (1060 daltons). MTX-1 from G. toxicus was found to be identical to the original MTX isolated from surgeonfish. The structure of MTX-2 has not been fully determined, but it appears to possess only one sulfate ester, compared to two in MTX-1. MTX-3 possesses two sulfate esters (48). MTX isolated from Caribbean G. toxicus clones has not been fully characterized structurally. MTXs have not been demonstrated to bioaccumulate is fish tissues, possibly due to their more polar structure. Thus, if MTX is involved in ciguatera poisoning, it may be implicated only in ciguatera poisonings derived from herbivorous fishes. Early hypotheses that MTX may be a metabolic precursor to CTX have not proven to be true (49).

The toxic potency of MTX exceeds that of CTX (LD50 0.05 mg/kg i.p. in mice). Its mode of action has not been fully elucidated. Its biological activity is strictly calcium dependent and causes both membrane depolarization and calcium influx in many different cell types. It was originally believed to be an activator of voltage dependent calcium channels (see 50 for review). However, voltage-dependent calcium channel antagonists can block MTX-stimulated calcium influx, but not MTX-induced membrane depolarization (51). Therefore, it appears that MTX-induced activation of voltage dependent calcium channels is a secondary effect of membrane depolarization. Despite numerous studies, the primary target of MTX has not yet been fully elucidated, although non-selective cation channels (52,53), and calcium activated chloride channels (54,55) have received recent attention. Calcium-release activated calcium (CRAC) channels, another proposed target, do not appear to be involved based on the failure of CRAC channel antagonists to inhibit MTX activity (56). Removal of the sulfate esters causes a significant drop in toxicity (57). MTX-induced calcium influx can be inhibited by PbTx and by MTX fragments, which suggests that both hydrophobic and hydrophilic domains of the molecule are necessary for target (58).

The definition of ciguatera is complicated by the fact that G. toxicus is, in fact, one member of an assemblage of benthic dinoflagellates, all of which produce toxins. Unlike the planktonic dinoflagellates, toxicity in the benthic coral reef dinoflagellate assemblage appears to be quite common. Among the dinoflagellates which co-occur with G. toxicus are Ostreopsis spp., Prorocentrum spp., Coolia spp. and Amphidinium spp. Each of these genera produces toxins targets which target different pharmacological receptors (Table 1). However, with the exception of toxins derived from Ostreopsis, the accumulation of most of these toxins in upper trophic levels of the coral reef community to concentrations which may impact human health has not been confirmed, and therefore their contribution to ciguatera remains equivocal. However, Ostroepsis has been proposed to be the primary dinoflagellate responsible for ciguatera in Puerto Rico, based on seasonal abundance of Ostreopsis vs G. toxicus in Peurto Rican waters (59). Ostreopsis produces ostreocin, an analog of palytoxin (Figure 8). Palytoxin has been confirmed as the causative agent in ciguatera-like poisonings from crab in the Pacific (60), mackerel (61), triggerfish (62), and sardines (clupeotoxism) (63). Palytoxin is a macrocyclic polyether toxin, characterized by a number of novel features including: a C115 straight chain incorporating many functionalities; a terminal primary amine that is important for bioactivity; an a,b-unsaturated amide, two conjugated diene systems, and a hemiketal (64). The complete structure of palytoxin was determined by Moore et al (65). Palytoxin poisoning may be distinguishable from ciguatera by its severity (high fatality rate) and unusual taste associated with the contaminated fish. The LD50 in rodents is 0.01 ó.25 mg/kg (66). The pharmacological target of palytoxin is Na+K+-ATPase, which pumps Na+ and K+ across the cell membrane against their electrochemical gradients, such that three Na+ ions are pumped out of the cell and two K+ ions are pumped into the cell for each ATP hydrolysed. In the presence of palytoxin, the pump is converted into an open channel that permits K+ efflux and influx of monovalent cations (Na+, NH4+, Cs+, Li+) along their electrochemical gradients. The palytoxin-induced pore appears to reside within the protein, possibly by stabilizing a channel made up of transmembrane segments of the protein when the pump is in its open state (67,68,69).

References:

34. Ahmed, FE. Seafood Safety. National Academy Press, Washington, D.C., 1991.

35. Vernoux, J-P. RL Lewis. Isolation and characterization of Caribbean ciguatoxins from the horse-eye jack (Caranx latus). Toxicon 35: 889-900, 1997.

36. Yasumoto, T, I Nakajima, R Bagnis, R Adachi, R. Finding of a dinoflagellate as a likely culprit of ciguatera. Bull Jap Soc Scient Fish 43: 1021-1026, 1977.

37. Legrand, AM. Ciguatera toxins: origin, transfer, through the food chain and toxicity to humans. In: B.Reguera, J Blanco, ML Fernandez, T Wyatt, eds. Harmful Algae. Santiago del Compostela, Spain: Xunta de Galacia and IOC. 1998, pp. 39-43,

38. Sheuer, PJ., W Takahashi, J Tsutsumi, T Yoshida. Ciguatoxin. Isolation and chemical properties. Science 155: 1267-1268, 1967.

39. Murata, M., AM Legrand, Y Ishibashi, M Fukui, T. Yasumoto. Structures and configurations of ciguatoxin from te moray eel Gymnothorax javonicus and its likely precursor from the dinoflagellate Gambierdiscus toxicus. J Am Chem Soc 112: 4380-4386, 1990.

40. Lewis, RJ, M Sellin, MA Poli, R Norton, JK MacLeod, MM Sheil. Purification and characterization of ciguatoxins from the Moray eel (Lycodontis javonicus, Muraenidae). Toxicon 29: 1115-1127, 1991.

41. Lewis RJ. MJ Holmes. Origin and transfer of toxins involved in ciguatera. Comp Biochem Physiol 106C: 615-628, 1993.

42. Legrand, AM., T Teai, P Cruchet, M Satake, K Murata, T Yasumoto. Two structural types of ciguatoxin involved in ciguatera fish poisoning in French Polynesia. In: B.Reguera, J Blanco, ML Fernandez, T Wyatt, eds. Harmful Algae. Santiago del Compostela, Spain: Xunta de Galacia and IOC. 1998, pp. 473-475.

43. Lewis, R.J, JP Vernoux, IM Brereton. Structure of Caribbean ciguatoxin isolated from Caranx latus. J Am Chem Soc 120: 5914-5920, 1998.

44. Dechraoui, M-Y, J Naar, S Pauillac, A-M Legrand. Ciguatoxins and brevetoxins, neurotoxic polyether compounds active on sodium channels. Toxicon 37: 125-143, 1999.

45. Yasumoto, T, R Bagnis, JP Vernoux. Toxicity of surgeonfishes II. Properties of the priniple water soluble toxin. Bull Jap Sci Fish 42: 359-365, 1976.

46. Murata, M, H Naoki, T Iwashita, S Matsunaga, M Sasaki, A Yokoyama,T. Yasumoto. Structure of maitotoxim. J Amer Chem Soc 115: 2060-2062, 1993.

47. Zheng, W, JA DeMattei, J-P Wu, JJ Duan, LR Cook, H Oinuma, Y Kishi. Complete relative stereochemistry of maitotoxin. J Am Chem Soc 118: 7946-7968, 1996.

48. Lewis, RL, MJ Holmes, PF Alewood, A Jones. Ionspray mass spectrometry of ciguatoxin-1, maitotoxin-2, and ñ3 and related marine polyether toxins. Nat Toxins 2: 56-63.

49. Lewis, RJ, NC Gillespie, CMJ Holmes, JB Burke, AB Keys, AT Fifoot, R Street. Toxicity of lipid-soluble extracts from demersal fishes at Flinders Reef, Southern Queensland. In: JH Choat, D Barnes, MA Borowitzka, JC Coll, PJ Davies, P Flood, BG Hatcher, D Hopley, PA Hutchings, D Kinsey, GR Orme, PF Sale, PA Sammarco, CC Wallace, C. Wilkinson, E Wolanski, O Billwood, eds. Proc Sixth Int Coral Reef Symp. Townsville: Exec Committee Sixth Int Coral Reef Symp 1988, Vol 3 pp. 61-65

50. Gusovsky, F., JW Daly. Maitotoxin: a unique pharmacological tool for research on calcium-dependent mechanisms. Biochem Pharmacol 39: 1633-1639, 1990.

51. Xi, D, FM Van Dolah JS Ramsdell. Maitotoxin induces a voltage dependent membrane depolarization in GH4 pituitary cells via activation of type L voltage dependent calcium channels. J Biol Chem 267: 25025-25031, 1992.

52. Estacion, M, HB Nguyen, JJ Gargus. Calcium is permeable through maitotoxin-activated nonselective cation channel in mouse L cells. Am J Physiol ñ Cell Physiol. 39: C1145-C1152, 1996.

53. Bielfeldackermann, A, C Range C Korbmacher. Maitotoxin (MTX) activates a nonselective cation channel in Xenopus laevis oocytes. Eu J Physiol 436: 329-337, 1998.

54. Young, RC, M McLaren, JS Ramsdell. Maitotoxin increases voltage independent chloride and sodium currents in GH4C1 rat pituitary cells. Nat Toxins 3: 419-27, 1995.

55. Martinez, M, C Salvador, JM Farias, L Vaca, LI Escobar. Modulation of a calcium-activated chloride current by maitotoxin. Toxicon 37: 359-370, 1999.

56. Daly, JW, J Leuders, WL Padgett, Y Shin, F Gusovsky. Maitotoxin induced calcium influx in cultured cells. Effect of calcium channel blockers. Biochem Pharmacol 50: 1187-1197, 1995.

57. Murata, M, F Gusovsky, M Sasaki, A Yokoyama, T Yasumoto. Effect of maitotoxin analogues on calcium influx and phosphoinositide breakdown in cultured cells. Toxicon 29: 1085-1096, 1991.

58. Konoki, KM, M Hashimoto, T Nonomura, M Sasaki, M Murata, K Tachibana. Inhibition of maitotoxin-induced Ca2+ influxin rat glioma C6 cells by brevetoxins and syntheticfragmants of maitotoxin. J Neurochem 70: 409-416, 1998.

59. Tosteson, TR. The diversity and origins of ciguatera fish poisoning. Puerto Rico Health Sci J 14: 117-128, 1995.

60. Alcala, AC, JS Garth, D Yasumura, T Yasumoto. Human fatality due to injection of the crab Demania reynaudii that contained a palytoxin-like toxin. Toxicon 26: 105-107, 1988.

61. Kodama, A., Y Hokama, T Yasumoto, M Fukui, SJ Manea, N Sutherland. Clinical and laboraotry findings implicating palytoxin as cause of ciguatera poisoning due to Decapterus macrosoma (mackerel). Toxicon 27: 1051-1053, 1989.

62. Fukui, M, M Murata, A Inoue, M Gawel, T Yasumoto. Occurrence of palytoxin in the triggerfish Melichthys vidua. Toxicon 25: 1121-1124, 1987.

63. Onuma, Y, M Satake, T Ukena, J Roux, S Chanteau, N Rasolofonirina, M Ratsimaloto, M., H Naoki, T Yasumoto. Identification of putative palytoxin as a cause of clupeotoxism. Toxicon 37: 55-65, 1999.

65. Hirata, Y, D Uenura, Y Yizumi. Chemistry and pharmacology of playtoxin, In: AT Tu. Handbook of natural toxins. Vol. 3. Marine Toxins and Venoms, New York : Marcel-Dekker, 1988, pp.241-258.

65. Moore, RE, G Bartolini, J Barchi, AA Bothner-By, J Dodok, J Ford,. Absolute stereochemistry of palytoxin. J Am Chem Soc 104: 3776-3779, 1982

66. Haberman, E. Palytoxin acts through the Na+/K+ ATPase. Toxicon 27: 1171-1187, 1989.

67. Redondo, J, B Fiedler, G Schneider-Bobis. Palytoxin-induced Na+ influx into yeast cells expressing mammalian sodium pump is due to formation of a channel within the enzyme. Mol Pharmacol 49: 49-57, 1996.

68. Scheinerbobis, G. H Schneider. Palytoxin-induced channel formation within the Na+K+ATPase does not require a catalytically active enzyme. Eu J Biochem 248: 717-723, 1997.

69. Scheinerbobis, G. Ion-transporting ATPases as ion channels. Archives of Pharmacology 357: 477-482, 1998.

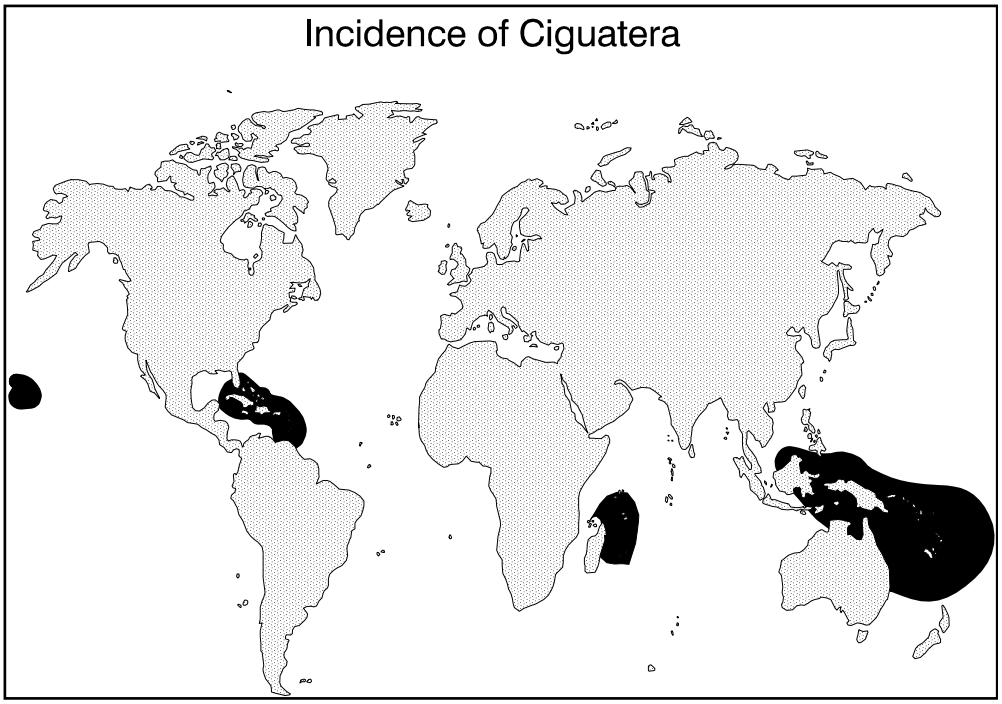
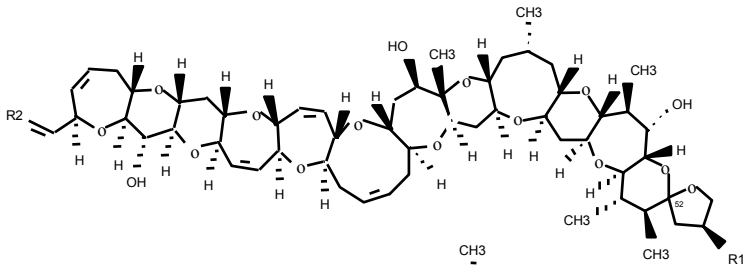


Figure 5. Worldwide distribution of ciguatera fish poisoning.

Type 1



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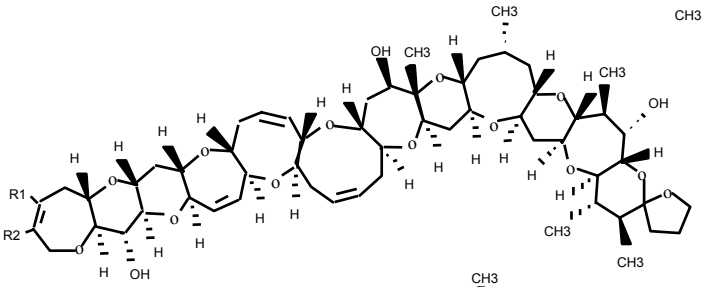

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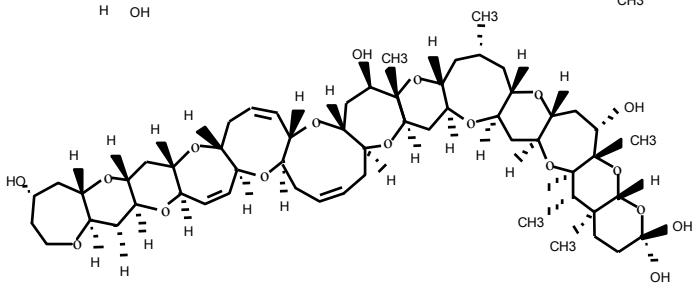

	Source	Type	R1	R2
CTX-1	fish	1	$HOCH_2CHOH$-	OH
CTX-2	fish	1	$HOCH_2CHOH$-	H
CTX-3	fish	1	$HOCH_2CHOH$-	H
CTX-4A	dino.	1	CH_2 = CH-	H
CTX-4B	fish,dino.	1	CH_2 = CH-	H
CTX-2A1	fish	2	OH	OH
CTX-3C	dino.	2	H	H
C-CTX-1	fish	3	-	-

Figure 6. Structures of the ciguatoxins in fish flesh and their putative dinoflagellate precurors. CTX-2 and -3 and CTX-4A and -4Bare epimeric pairs around the C52 spiroketal.

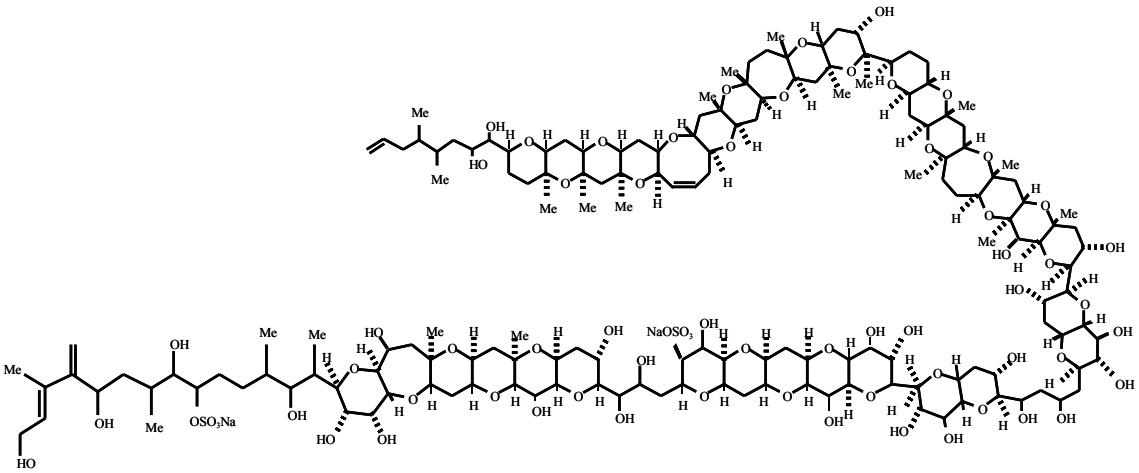

Figure 7. Structure of MTX.

Table 1. Toxins produced by benthic coral reef associated dinoflagellates.

Species	Toxin	Pharmacological Target
Gambierdiscus toxicus	ciguatoxin maitotoxin	voltage dependent sodium channel (?) calcium dependent
Coolia monotis	coolia toxin	unknown
Ostreopsis spp.	ostreocin	Na^+K^+ ATPase
Prorocentrum spp	okadaic acid	ser/thr protein phosphatases
Amphidinium spp.	amphidinilides	unknown (antifungal)

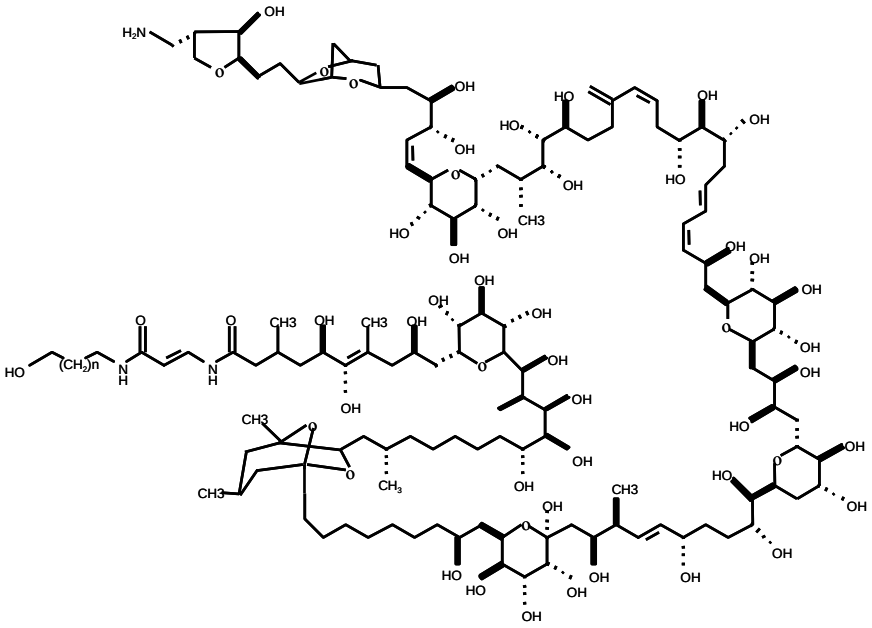

Figure 8. Structure of palytoxin.